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THE EVOLUTION OF PINK: MORPHOLOGICAL AND GENETIC VARIATION AMONG THREE *LITHOPHRAGMA* (SAXIFRAGACEAE) SPECIES

Rebecca A. Hufft and James E. Richardson[1]
Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, CA 95064
hufft@darwin.ucsc.edu

Abstract

Prior molecular work using ITS and chloroplast sequence data revealed that the endemic *Lithophragma trifoliatum* and the broad-ranging *L. parviflorum* form a tight clade with a third species, *L. affine*. We used AFLPs to assess the fine scale relationship of these three species from populations where their distributions overlap in northern California. Our results revealed two groups of *L. trifoliatum*, one nested within a group of *L. affine* and *L. parviflorum* and the other grouped with other populations of *L. parviflorum*, contrary to predictions based on plant morphology alone. The morphological pattern was not supported by the molecular data, suggesting that pink flowers evolved more than once, concurrently with other floral traits such as size and nectary length. It is also possible that this pattern is due to recent evolution or gene flow between the two color morphs. The possible ecological importance of these differences in floral traits (e.g., for pollination) warrants further study, as well as the extent to which these populations are reproductively isolated.

Key Words: Amplified fragment length polymorphisms (AFLPs), endemic, floral traits, genetic variation, *Lithophragma*, Saxifragaceae.

Floral morphology has been found to be important in reproductive success (e.g., Galen 1989; Herrera 1993; Guitian et al. 1997), pollinator specialization (Muchhala 2003) and isolation between plant species (e.g., Bradshaw Jr. et al. 1998; Ellis and Johnson 1999; Fulton and Hodges 1999). Floral traits may dictate reproductive success by mediating attractiveness to pollinators. In this way, individual pollinators may exert strong directional selection for particular floral syndromes (Campbell et al. 1997). In part because they often play a role in reproductive isolation, floral traits are also used to differentiate closely related species. Yet, are these floral traits meaningful in explaining the genetic relatedness among populations and species? Floral morphology alone can be phylogenetically deceptive due to convergent evolution and the gene flow and hybridization that can result from shared pollinators.

The processes of diversification and speciation remain important problems in evolutionary biology because species arise through many genetic mechanisms and their relative importance among different taxa is unresolved (Hewitt 2001). Diversification in plants is particularly intriguing because they may speciate through hybridization and exhibit reticulate evolution. Incomplete reproductive isolation can limit diversification, but it also may maintain a greater range of floral morphologies, as intermediate morphotypes would be maintained due to gene flow and hybridization. Hybridization can result in genetic variation, which provides the raw material for rapid adaptation and can play an important role in evolutionary diversification (Rieseberg 1997; Arnold et al. 1999; Rieseberg et al. 2000).

The genus *Lithophragma* (Saxifragaceae) provides an opportunity to evaluate how floral morphology and hybridization contribute to the diversification of plant taxa. The genus has ten named species that differ in floral morphology, hybrid history and geographic range (Taylor 1965). Three of the species within this genus, *L. affine* A. Gray, *L. parviflorum* (Hook) Torrey & A. Gray, and *L. trifoliatum* Eastw. form a tight clade and represent the extremes in geographic distributions within the genus (Taylor 1965; Nicholls and Bohm 1984; Soltis et al. 1992; Kuzoff et al. 1999). Hybridization has been suggested among these three species (Taylor 1965). In addition, results from a phylogeny based on internal transcribed spacer (ITS) sequences of ribosomal DNA showed these species as a paraphyletic group (Kuzoff et al. 1999). The authors thought the most compelling reason for this is that the putative species are not distinct lineages. In the literature, there is disagreement regarding their specific status: Taylor (1965) lists them as three separate species, while the Jepson Manual (Hickman 1996) lists two species, *L. affine* and *L. parviflorum* vars. *parviflorum* and *trifoliatum*.

Although existing molecular studies have shown these three species to be genetically very similar, they differ in several morphological traits, including some (i.e., floral scent and color; see Table 1) that are unique

[1] Current address: Biosystematics, Wageningen University and Research Centre, P.O. Box 9101, 6700 HB Wageningen, the Netherlands.

TABLE 1. SUMMARY OF SPECIES DESCRIPTIONS. INFORMATION IN TABLE FROM THE JEPSON MANUAL (HICKMAN 1993) AND TAYLOR (1965). The Jepson Manual (Hickman 1993) lists *L. trifoliatum* as *L. parviflorum* var. *trifoliatum* and *L. parviflorum* as *L. parviflorum* var. *parviflorum*.

Species	Flower color	Floral shape	Leaf shape	Floral scent	Ploidy (2n)	Distribution
L. affine	White	Hypanthium obconic, inflated above	Shallowly lobed	none	14, 21, 28	Coastal mountains of CA, a few specimens in outer central Sierra Nevada foothills. Open well-drained grassy clearings or open bluffs in oak or coniferous-oak woodlands from sea level to 2150 m
L. parviflorum	Generally white, some pink	Hypanthium long-obconic, 2× longer than wide	Deeply lobed	none	14, 21, 28, 35	W. NA (BC, WA, ID, MT, OR, WY, SD, CA, NV, UT, CO); Habitat extremely variable, seacoast bluffs to open gravelly prairielands, to subalpine regions up to 3050 m elevation
L. trifoliatum	Pink	Hypanthium long-obconic, 3–4× longer than wide	Deeply lobed	fragrant	28	Restricted to western slope of the Sierra Nevada, with center in Butte Co., CA. In igneous-derived scabland in oak-coniferous woodland from 60–600 m elevation

within the genus. Northern California is the only place in the range of this genus where plants with pink flowers occur. Along with these pink flowers are other unusual traits for this genus, such as much longer petals and hypanthia (see Table 1). There are also populations that have larger white flowers, resembling *L. parviflorum*, but that have leaf morphology more similar to *L. affine*. Populations with mixed traits (generally labeled *L. parviflorum*) in this region led Taylor (1965) to conclude that there was hybridization in this region.

This species complex provides an opportunity to study two central questions regarding the diversification of plant taxa: 1) Do these populations of putative species differ consistently in the floral traits that are used to distinguish the species? 2) Do patterns of floral morphology inform our understanding of phylogenetic relationships among these species? Specifically, does the unique pink color correlate with other floral traits that differ consistently among species? If the pink flowers have evolved only once and are an important reproductive isolating mechanism among these species, we expect the populations with pink flowers to be more closely related to each other than to populations with white flowers and for there to be limited gene flow between populations of different flower color.

MATERIALS AND METHODS

Geographical Distribution of *Lithophragma*

Lithophragma (Saxifragaceae) is an herbaceous perennial genus with a broad geographical distribution from southern California to southern British Columbia and from the west coast of North America to South Dakota (Taylor 1965). The genus is thought to have originated within California (Taylor 1965), where most taxa and the most basal taxa occur (Taylor 1965; Soltis et al. 1992; Kuzoff et al. 1999).

Molecular data indicate that *L. affine*, *L. parviflorum*, and *L. trifoliatum* are so similar that the limits of the species are uncertain with respect to both the chloroplast and nuclear markers that have been used (Soltis et al. 1992; Kuzoff et al. 1999). These three species may, therefore, not be distinct lineages. Nonetheless, these taxa differ in a variety of morphological traits and geographical distribution, with more differentiation occurring among populations and species in California (near the purported center of the distribution) than in more northerly populations.

Lithophragma affine is primarily restricted to the coastal mountains of California from Humboldt County to Santa Barbara County (Taylor 1965), but a few specimens have been found in the foothills of the central Sierra Nevada, in Tuolumne, Stanislaus, and Amador counties (CalFlora Occurrence Database; UC, Berkeley Jepson Herbarium). Taylor (1965) described *L. affine* as very polymorphic due to environmental variability and population isolation caused by the topography of the region.

Lithophragma parviflorum is the most widely distributed species in the genus. It ranges from southern California to southern British Columbia and from the west coast of North America to South Dakota. Across its wide range, *L. parviflorum* shows great morphological variation.

Lithophragma trifoliatum has the narrowest distribution in the genus and is restricted to the western slope of the Sierra Nevada. Taylor (1965) considered this species closely related to *L. parviflorum*. His data indicated that this species represented a sterile derivative of *L. parviflorum* that was persisting through vegetative reproduction in a small geographic area. (However, field-collected seeds have germinated in the greenhouse (Hufft unpublished data), but the extent of their viability across all populations is not known.) More recent phylogenies have shown that *L. trifoliatum* is part of the *L. parviflorum/affine* clade, but its exact relation to the other two species is not known (Soltis et al. 1992; Kuzoff et al. 1999).

Sampled Populations

The distributions of all three species, which overlap in northern California, provide an opportunity to study local phylogeographic patterns of this complex, which have been unresolved in the larger scale phylogeographic work that has been done on this genus. Sixteen populations, along with several roadside collections, were located in spring 2001 and 2002 in Mendocino, Tehama, Butte, Plumas, and Lassen Counties (Fig. 1). Populations were chosen within the zone of overlap in northern California that represent the morphological diversity of the three species (Table 2). Populations were identified to species based on the floral characteristics that define them in Jepson (Hickman 1994) and verified by a local expert, Vern Oswald. Plants were haphazardly selected and were chosen at least two meters from the nearest plant used to ensure that they were distinct individuals and not growing from the same underground bulbils.

Floral Morphology

Although these species have similar floral structures, relative to other species in the genus (Kuzoff et al. 2001), variation in floral traits are used to distinguish among the three species (Taylor 1965; Hickman 1996). Nine morphological traits were measured on field-collected flowers: average petal length, average petal width, corolla gap, floral length, tip to nectary, short angle, average nectary depth, nectary length, and average diaganol (Fig. 2). The second flower from each plant was collected from all study sites (Table 2) and stored in 70% ethanol. Floral traits were measured using a microscope (Wild M8 microscope) fitted with an ocular micrometer. For these nine traits, 144 flowers were analyzed from 14 populations (Table 2) using Principal Components Analysis (PCA).

Flower color was not included in the analysis because it is not a quantitative trait and only one color (white or pink) occurred within a population. Instead, flower color was used as a grouping variable in the various analyses to determine its usefulness in distinguishing among the species.

We used discriminant analysis to determine if flower color, species, or molecular group (based on AFLP data, see below) better differentiated these individuals based on floral morphology (SAS 6.12 1996). Half of the samples in each group were randomly selected to create the discriminant function, with the other half used to test the model. The proportion of test samples classified correctly provided a quantitative measure of the ability of each grouping variable to accurately distinguish among these individuals. Estimates of pairwise population morphological distances were calculated with discriminant analysis (SYSTAT 10.2 2002) for comparison with estimates of genetic distance (see below).

Pollinator Observations

We performed pollinator observations at six sites in spring 2002 (1 *L. affine*, 1 white *L. parviflorum*, 1 pink *L. parviflorum*, and 3 *L. trifoliatum*). We chose plants haphazardly and observed all plants within a 1-m quadrat (number of plants per observation=1–13) for 30 min. We performed a total of 112 observation periods (15 *L. affine*, 61 *L. parviflorum*, and 35 *L. trifoliatum*). We recorded the number of flowers within a quadrat, the number and identity of pollinator visitors and the number of flowers visited.

Amplified Fragment Length Polymorphisms (AFLPs)

Gene flow within and among populations and patterns of relatedness among individuals and populations was evaluated using genetic fingerprinting (amplified fragment length polymorphisms [AFLPs; Vos et al. 1995] that are predominantly nuclear). DNA was extracted using the method from Doyle and Doyle (1987). Following standard protocols, AFLPs were analyzed (Applied Biosystems manuals 1997). A total of 158 individuals from 16 populations (Table 2) were scored for the presence of 216 markers from two ABI AFLP primers, CAT-ACT (blue) and CAG-AAG (green). Data were analyzed with an AMOVA using ARLEQUIN (Schneider et al. 2000), a Principle Coordinate Analysis (PCoA) using the R Package (Casgrain 2004) and UPGMA using PAUP 4.0b10 (Swafford 2001) and visualized in TreeView (Page 2001). Pairwise population differences were calculated, a one-way AMOVA was performed to measure among population variation and a hierarchical AMOVA was performed to partition the variance into species and floral color effects. The relationship between floral morphol-

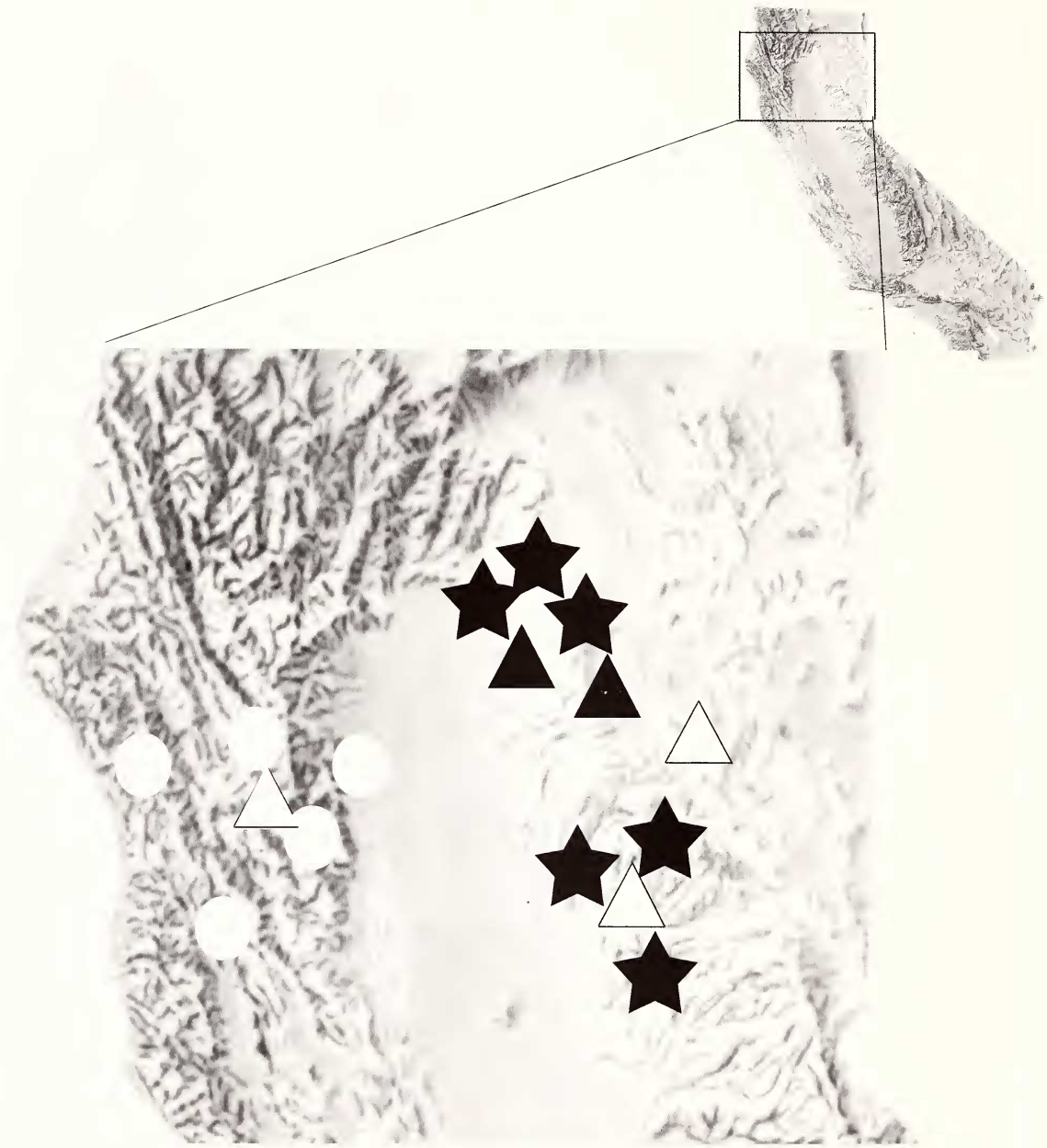

FIG. 1. California field sites, as numbered in Table 2. White circles = *L. affine*, white triangles = white-flowered *L. parviflorum*, black triangles = pink-flowered *L. parviflorum*, and black stars = *L. trifoliatum*.

ogy and genetic relatedness was first assessed by comparing the output of the PCA and PCoA. The quantitative floral morphology data and AFLP data were then statistically compared using a regression of the AFLP genetic distances and the floral morphology distances, to test for a positive relationship between genetic and floral distances from the discriminant analysis. A log transformation was performed on floral distances to normalize data prior to performing the linear regression.

RESULTS

Floral Morphology

Results of the PCA suggest that populations with pink flowers (*L. parviflorum* and *L. trifoliatum*) can be distinguished from populations with white flowers (*L. parviflorum* and *L. affine*) to some degree but the measured floral traits exist along a continuum (Fig. 2). Axis 1 and Axis 2 explained 46% and 23% of the variation, re-

TABLE 2. SPECIES, FLOWER CCOLOR AND NUMBER OF SAMPLES UUSED FOR THE MORPHOLOGICAL AND GENETIC ANALYSES FOR EACH POPULATION. [a] Not used in AMOVA analyses.

Population	Species	Flower Color	Flower Count	AFLP count
1. Alder	Affine	White	1	6
2. Big Oak	Affine	White	14	10
3. Hwy101	Affine	White	6	1[a]
4. Mendocino1	Affine	White	9	11
5. Mendocino2	Affine	White	5	10
6. Feather Falls	Parviflorum	White	10	14
7. Hwy70	Parviflorum	White	19	10
8. Plasket Meadows	Parviflorum	White	0	2
9. Dye Creek	Parviflorum	Pink	1	9
10. Hogsback	Parviflorum	Pink	60	25
11. Forbestown	Trifoliatum	Pink	0	3
12. Hog Lake	Trifoliatum	Pink	1	12
13. Hwy 36	Trifoliatum	Pink	0	3
14. MilsapBar	Trifoliatum	Pink	3	17
15. North Table MT	Trifoliatum	Pink	10	14
16. Shingletown	Trifoliatum	Pink	0	11
		Total	**139**	**158**

spectively (Table 3). Most populations did not fall out as tight clusters (data not shown). The white and pink flowered individuals separate out mainly along Axis 2. The traits that have the strongest influence on axis 2 are length of nectary (with an eigenvector value of 0.556), nectary depth (0.443) and corolla gap size (0.519). Flower color was a very good grouping variable in the DFA, with high classification rates for both colors (Table 4). Although, *L. parviflorum* and *L. affine* also showed high classification rates, *L. trifoliatum* proved to be a very bad grouping variable, with the majority of *L. trifoliatum* individuals being classified as *L. parviflorum* (Table 4). The molecular groups had a higher total misclassification of individuals (35%) than either color (13%) or species (26%).

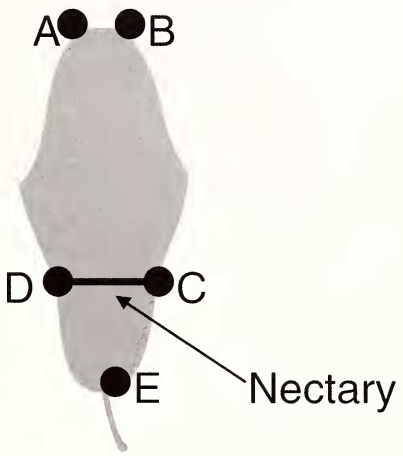

FIG. 2. Diagram illustrating floral measurements. Corolla gap = distance between A and B, flower length = midpoint of A and B to E, tip to nectary = midpoint of A and B to the midpoint of C and D, short angle = B to C, average diagonal = A to C and B to D, average nectary depth = height of nectary, and nectary length = C to D.

Pollinator Observations

We recorded 128 insects visiting 324 flowers. These preliminary observations revealed that although the pink populations received more visits (1.15 pollinators/observation period vs. 0.56 for white populations), generalists (Bombyliid flies and solitary bees) were visiting all of the plants, indicating the possibility of gene flow between the color morphs. Unlike previous studies of *L. parviflorum* (Thompson and Pellmyr 1992; Thompson 1999; Thompson and Cunningham 2002), the specialist *Greya politella* was found at only one site (6. Feather Falls).

AFLPs

The results of the PCoA are shown in Figure 4, with individuals labeled by species and flower color. *Lithophragma trifoliatum* is split into two groups along axis 2, and the majority of samples with pink flowers are clumped along the same half of axis 1. Additionally, the AFLP results can be seen in the UPGMA phenogram (Fig. 5). Although, populations mostly group together, there is very low resolution of the relationships among populations. Population 15 (North Table Mountain) appears to be the most derived. Although not strongly supported, Population 14

TABLE 3. RESULTS OF PRINCIPAL COMPONENT ANALYSIS OF FLORAL MORPHOLOGY. Variance extracted on the first three components (a) and eigenvectors of floral characters (b).

A.

Component	Eigenvalue	% of Variance	Cum.% of Var.
1	4.181	46.450	46.450
2	2.055	22.834	69.285
3	0.818	9.085	78.369

B.

Character	Eigenvector 1	Eigenvector 2	Eigenvector 3
Average petal length	−0.3814	−0.1771	0.1858
Average petal width	−0.3831	−0.1858	0.1583
Corrolla gap	−0.1280	0.5185	−0.5339
Floral length	−0.3623	0.1151	0.1068
Tip to nectary	−0.4211	−0.1961	−0.0721
Short angle	−0.4184	−0.2448	−0.0660
Average nectary depth	−0.1032	0.4426	0.7313
Nectary length	−0.1388	0.5561	0.0728
Average diagonal	−0.4216	0.2202	−0.3070

(Milsap Bar) is more closely related to the two geographically closest populations (6. Feather Falls and 7. Hwy 70) than to other *L. trifoliatum* populations.

The one-way AMOVA results revealed significant genetic differentiation among populations, with 35.9% of the variation partitioned among the populations. The pairwise genetic distances are given in Table 4. Of the 105 comparisons, 6 were not significant. A nested ANOVA of genetic distances grouped by comparisons within species versus comparisons among species revealed that there was no difference between within versus among species comparisons (F-ratio = 0.686, df = 1, P = 0.409), meaning that overall distances between populations of different species were not different from distances of populations of the same species. However, there were significant differences in the comparisons of distances among species and the distances within species (F-ratio = 2.898, df = 4, P = 0.024), with pairs involving *L. trifoliatum* having the largest within species distances and those involving *L. affine* having the smallest. The *L. trifoliatum*-*L. parviflorum* group was the largest among species distance. However, distances between populations of the same color were more similar than populations of different color (Pooled Variance t = 2.757, df = 151, P = 0.007). No relationship was found between the pairwise genetic and morphological distances (b = −0.022, P = 0.222, adjusted r^2 = 0.007). The power of this test to detect a positive slope as small as 0.05 was 0.77.

DISCUSSION

Currently defined species within this group (Hickman 1996; Taylor 1965) do not appear much differentiated by floral morphology. The traits that seem to be most important in separating individuals in the PCA are corolla gap, nectary length and nectary depth, all traits that could be important in pollinator preference (Campbell et al. 1997; Fulton and Hodges 1999). In addition, floral color can be an important cue in pollinator discrimination (Wilson and Stine 1996) and also appears to distinguish two main groups of individuals when they are plotted onto the first two principal components. However, the three species are not segregated into discrete groups by the principal components. The predominance of generalist pollinators in this region would suggest that other, possibly neutral mechanisms, are maintaining floral variation. It has been hypothesized that diversity of ovary position in species of *Lithophragma* may be the result of modifications in one or a few genes (Kuzoff et al. 2001). This could also be the cause of the variation in other floral traits, like those measured here.

Using genetic markers to analyze these groups, the three species clump together indicating they are closely related. The degree of overlap suggests these are not distinct species. Location is important (i.e., populations clump together indicating individuals within a population are closely related), but flower color also has some genetic component (i.e., clumping of pink and white flower color along Axis 1, Fig. 4). Larger genetic distances between *L. trifoliatum* populations help explain the separation of this species into two groups in the PCoA. This could indicate more variation within populations or multiple lineages. This was also supported in the discriminant function analysis of morphological traits, where *L. trifoliatum* was not found to be a viable group. Flower color was not as good a grouping variable for the genetic relationships as it was for the floral morphology (see separation of pink

TABLE 4. DISCRIMINANT FUNCTION ANALYSIS OF FLORAL MORPHOLOGY GROUPED BY SPECIES, COLOR AND MOLECULAR GROUP. Molecular groups were based on AFLP results: Group 1 = 1.Alder, 3.Hwy101, 7.Hwy70; Group 2 = 6.Feather Falls, 14.Milsap Bar; Group 3 = 2.Big Oak, 4.Mendocino1, 5.Mendocino2; Group 4 = 15.North Table Mountain; Group 5 = 9.Dye Creek, 10.Hogsback, 12.Hog Lake. The total-sample standardized canonical coefficients are shown, along with the percent classifications for the testing samples. [a]Total error=26.05%. [b]Total error=13.25%. [c]Total error = 35.38%.

Total-Sample Standardized Canonical Coefficients

Species:	Petal length	Petal width	Corrola gap	Flower length	Tip to nectary	Short angle	Nectary depth	Nectary length	Diagonal
CAN1	0.301	−0.120	−0.104	0.898	1.748	−1.99	0.173	−1.050	0.381
CAN2	−0.991	0.683	1.003	0.925	−5.547	5.713	−0.464	−0.027	−0.299
Color:									
CAN1	−00.015	0.044	−.492	0.838	0.346	0.474	−0.196	−.0.737	−0.292
Molecular:									
CAN1	0.337	0.240	−0.173	0.848	−0.177	0.765	0.001	−1.271	0.030
CAN2	0.824	−0.996	0.653	0.687	0.657	−0.914	0.398	−0.152	0.046
CAN3	−00.790	0.371	−0.033	1.233	1.502	−1.225	−0.391	0.864	−0.915
CAN4	0.281	0.755	0.290	−0.332	−0.085	−0.635	−0.410	−0.109	0.802

Percent Classified into Species:

From Species:	*L. parviflorum*	*L. trifoliatum*	*L. affine*	% error[a]	No. of samples
L. parviflorum	84.09	13.64	2.27	15.91	44
L. trifoliatum	57.14	28.57	14.29	71.43	7
L. affine	31.25	0	68.75	31.25	16

Percent Classified into Color:

From color:	Pink	White	% error[b]	No. of samples
Pink	91.89	8.11	8.11	37
White	19.35	80.65	19.35	31

Percent Classified into Molecular Group:

From group:	1	2	3	4	5	% error[c]	No. of samples
1	46.15	23.08	15.38	0	15.38	53.85	13
2	33.33	33.33	33.33	0	0	66.67	6
3	21.43	7.14	64.29	0	7.14	35.71	14
4	0	0	0	0	100	100.00	5
5	0	0	6.67	3.33	90.00	10.00	30

TABLE 5. PAIRWISE POPULATION DIFFERENTIATION EXPRESSED BY Φ_{ST} (EXCOFFIER ET AL. 1992). Bold values are not significant. Population names refer to population number and species (Aff = *L. affine*, Par = *L. parviflorum*, Tri = *L. trifoliatum*). For *L. parviflorum*, flower color is also noted (P = pink, W = white).

	1Aff	2Aff	4Aff	5Aff	6ParW	7ParW	8ParW	9ParP	10ParP	11Tri	12Tri	13Tri	14Tri	15Tri
2Aff	0.412													
4Aff	0.391	0.202												
5Aff	0.316	0.236	0.218											
6ParW	0.545	0.429	0.263	0.404										
7ParW	0.296	0.296	0.198	0.265	0.188									
8ParW	0.314	0.365	0.279	0.337	0.508	**0.105**								
9ParP	0.499	0.350	0.224	0.344	0.369	0.253	0.464							
10ParP	0.397	0.286	0.169	0.245	0.213	0.185	0.317	0.094						
11Tri	0.463	0.359	0.125	0.327	**0.062**	**0.062**	**0.271**	0.335	0.147					
12Tri	0.601	0.443	0.351	0.417	0.486	0.334	0.600	0.354	0.213	0.552				
13Tri	0.423	0.323	0.128	0.268	0.409	0.158	**0.257**	0.185	**0.036**	0.338	0.326			
14Tri	0.579	0.476	0.348	0.462	0.259	0.258	0.569	0.502	0.306	0.328	0.568	0.525		
15Tri	0.649	0.488	0.369	0.487	0.561	0.424	0.660	0.449	0.295	0.590	0.626	0.528	0.613	
16Tri	0.524	0.299	0.237	0.314	0.408	0.301	0.514	0.241	0.178	0.440	0.363	0.271	0.504	0.456

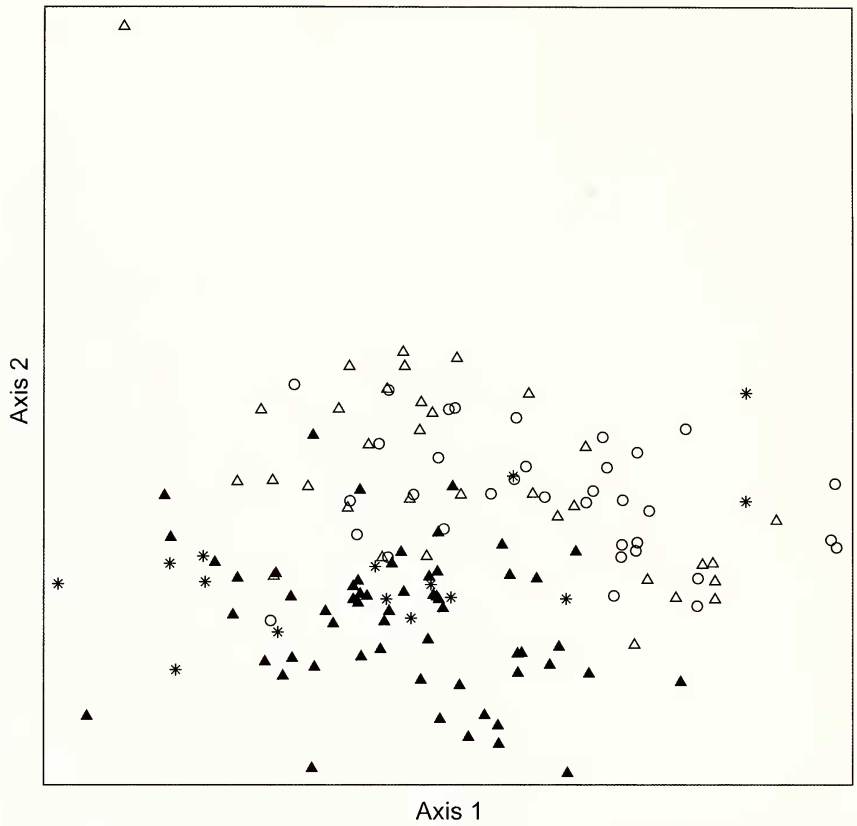

FIG. 3. Principal Component Analysis of floral morphology data labeled by species and flower color. ○ *L. affine*, white; △ *L. parviflorum*, white; ▲ *L. parviflorum*, pink; * *L. trifoliatum*, pink.

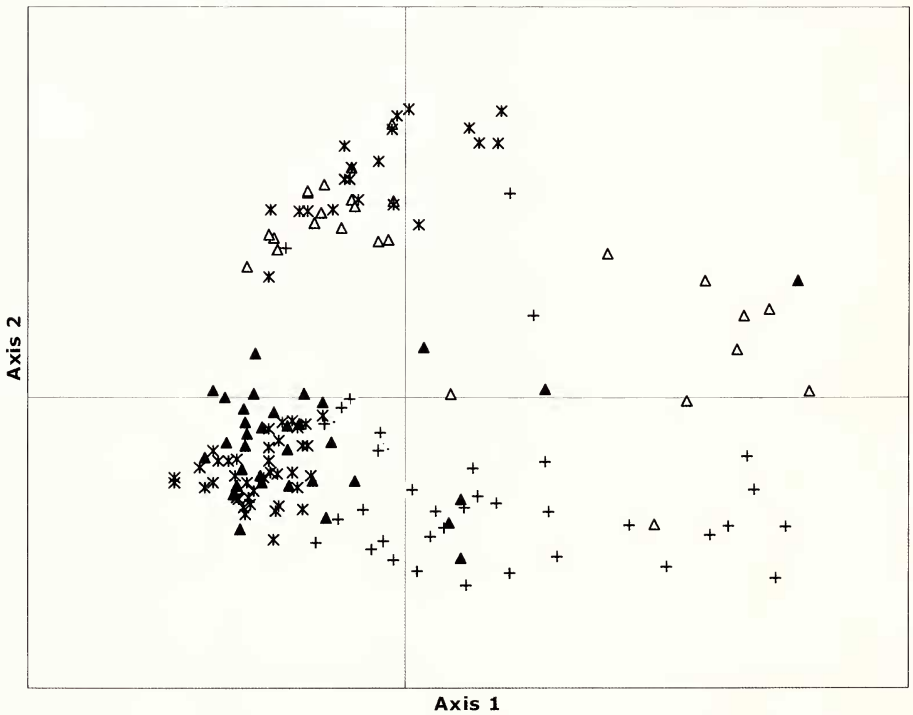

FIG. 4. Principal Coordinate Analysis of AFLP data. + *L. affine*, white; △ *L. parviflorum*, white; ▲ *L. parviflorum*, pink; ✳ *L. trifoliatum*, pink.

FIG. 5. UPGMA phenogram of AFLP data. Bars to the right of figure indicate flower color (black = pink) and species (black = *L. trifoliatum*, gray = *L. parviflorum*). White flowers and *L. affine* are indicated by spaces between the bars. * mark bootstrap values greater than 70%.

into two main groups in Fig. 4, and lack of association in Fig. 5). This could be because the pink morph has evolved more than once, it evolved recently or there is gene flow between the two color morphs. If pollinators were a strong isolating mechanism we would expect less overlap among the species and between individuals of different flower color in the AFLP data, indicating less gene flow. The lack of a relationship found between the molecular and morphological

data could be due in part to shared pollinators. However, it is also possible that very strong selection has resulted in the morphological divergence we see despite the molecular evidence of gene flow. The floral traits are thought to be genetically inherited, but a common garden experiment is necessary to separate the environmental component that could be responsible for some of the variation in the data (although no obvious habitat variation is known among the populations).

The three taxa studied here differ in the size of their geographic ranges, which might influence the morphological variation observed, as you would expect species with larger ranges to have more morphological variation due to the increased environmental variation across their range. An additional aspect of this work was to identify genetic and ecological differences between a narrow endemic and its broad ranging relatives. It is expected that rare plants have low phenotypic variability (Kruckeberg and Rabinowitz 1985), but this is not always the case (Guitian et al. 1997). Given the narrow distribution of *L. trifoliatum* relative to its two sister species studied here, the expectation would be for it to show less variation in phenotypic traits than *L. affine* and *L. parviflorum*. However, all three species show similar amounts of variation for each individual trait measured (data not shown). It is possible that the morphological variation seen over the range of this clade is not due to differences among three species, but rather is just variation within one or two species. In agreement with previous work in this system, this research shows there is strong evidence that these are not three distinct lineages. The addition of the morphological data also supports previous molecular work that *L. trifoliatum* may not be a true, distinct species.

In order to better understand why these species show more variation in morphology than at neutral molecular markers, the relative importance of selection and local adaptation must be determined. More pollinator observations, preference trials and estimates of pollinator travel distance will help us better understand the role of biotic selection on these species. Sorting out the amount of gene flow and the mechanisms responsible for the maintenance of floral variation will also aid our understanding of the roles of selection versus drift in creating this variation in floral morphology. Exploring these diversification mechanisms will give us insight into their role in creating and maintaining biodiversity on a broader scale.

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